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PRINCIPAL INVESTIGATOR: Anne E. Peaston

CONTRACTING ORGANIZATION: The University of Adelaide
Adelaide 5000 Australia

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Anne Peaston

E-Mail: anne.peaston@adelaide.edu.au

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14. ABSTRACT
The purpose of this project is to investigate molecular events occurring in the preclinical stages of mammary cancer. Specifically, the project investigates the intersection between the development of genome demethylation, retrotransposon transcriptional activity, and retrotransposon-driven transcription of cellular genes in an engineered mouse model of mammary cancer. During the last 12 months, mouse breeding for the project was completed and collection of material for planned molecular analyses was delayed but is now almost complete. Preparation of RNA and DNA for molecular library construction is nearly complete, construction of molecular libraries is underway in Australia and with my collaborator Dr Edwards at Washington University in St Louis. Library analyses have been initiated in Dr Edwards' laboratory. We believe that the eventual findings will provide insights into understanding the role of genome hypomethylation and expression of retrotransposons in cancer ontogeny, and may impact cancer prevention in the future.

15. SUBJECT TERMS
Breast cancer, epigenetic, DNA methylation, retrotransposon, preclinical cancer development, deep sequencing.

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Table of Contents

Introduction

This project is designed to address the subject of mammary cancer development. The purpose of the project is to investigate molecular events occurring in the preclinical stages of mammary cancer; the results may lead to insights into cancer prevention in the future. Specifically, the project investigates the intersection between genome demethylation, retrotransposon transcriptional activity, and retrotransposon-driven transcription of cellular genes. Retrotransposon promoters are well recognized to function as alternative promoters for different cellular genes, generating chimeric transcripts that may or may not function in the same way as transcripts from the regular gene promoter. Transcriptional activation of retrotransposons is strongly linked with their CpG DNA methylation, and global genomic demethylation is one of the commonest molecular changes in malignancies. The project tests the hypothesis that, in preclinical stages of tumour development, progressive genomic demethylation leads to increased transcriptional activity of retrotransposons and this, in turn, leads to transcription of otherwise silent genes, potentially setting up molecular conditions that favour cancer development. We developed a genetically engineered mouse model in which a specific mammary cell population is fluorescently marked upon initial transcriptional activation of the SV40 large T antigen (SV40Tag) oncogene. SV40Tag is transcriptionally activated during pregnancy and lactation, and the mice are predisposed to develop mammary cancer after 3 pregnancies and lactations. Using this model, populations of marked cells can be collected for integrated analysis of gene expression, promoter usage, and DNA methylation after defined amounts of exposure to SV40Tag during different stages of preclinical cancer development.

Body

Dr. Peaston is the PI of this project and also maintains an academic appointment in the new School of Animal and Veterinary Sciences (SAVS) at The University of Adelaide in Australia involving teaching, administration and clinical (veterinary) roles. The principal roles of Dr. Peaston as a PI in this project are to establish mouse breeding and production colonies for the project, to collect and process mammary samples from the mice, forward samples for analysis to her collaborating colleagues at Washington University in St Louis, and perform experiments testing analysis results. For this project, Dr Peaston has completed the mouse production planned for the project, and has nearly completed collecting and processing mammary samples from the mice.

Since the previous report, there were further difficulties with mouse production and breeding programs. Although relatively minor, they contributed to ongoing delays in completing the majority of the mouse work for the project. However the mouse production and breeding for the 3 biologic replicates of the project is now complete.

Temporary closure of the flow cytometry facility for upgrading created another setback in collection of mouse mammary cells, but collection is now complete for 2 biologic replicates and almost complete for the third.

DNA and RNA preparation have recently been completed for biologic replicates 1 and 2. DNA from replicate 1 has been shipped to Dr Edwards at Washington University in St Louis for library construction and methyl-MAPS analysis. DNA replicates 2 and 3 will be shipped within the next few weeks.

The arrangements for RNA library construction and analysis have changed. Continuing staff and management changes at The Jackson Laboratory made the original plan for RNA library construction and analysis there economically less attractive, and both library construction and informatics analysis less feasible as Jackson's work priorities have changed. However, high quality library construction is now available at a reasonable price locally in Adelaide, at the Austalian Genome Research Facility (http://www.agrf.org.au/). RNA from all three biologic replicates will be batch-processed for library construction and paired end sequencing once cell collection and RNA extraction for the third replicate is complete, we estimate this will be in late October or early November 2013. The platform used for reporting sequencing results is compatible with data forms used by Dr Peaston's collaborator Dr. John Edwards at Washington University in St Louis and the data will be sent to him for analysis. His group has developed the experience and capability to perform RNA library analysis together with the DNA data, thus centralizing the project's data analysis to the one group. It is not anticipated that the change in source for library construction and analysis will have a significant effect on the project outcomes.

Key Research Accomplishments

We are acutely aware that this report contains no molecular data from the project, however, we are confidant that this deficit will be corrected over the coming months.

We have been granted a 12-month no-cost extension of the grant, and in the extension period we plan to accomplish:

- complete preparation of biologic replicates 1-3 of the the RNA-seq libraries and CAGE libraries (Peaston), and methyl-MAPS DNA libraries (Edwards)
- complete the preliminary analyses of all three biologic replicates of the libraries (Edwards)
- conduct RT-PCR assessment of transcripts of interest (Peaston)
- conduct bisulfite sequencing assessment of DNA loci of interest (Peaston)
- Consider final approaches to data analysis/integration and publication planning (Peaston and Edwards)
- prepare to report results at the Baltimore and/or San Antonio breast cancer meetings (Peaston, Edwards)
- prepare a report for publication of the results of this study (Peaston, Edwards)
- Organize continued contact between Drs Edwards and Peaston for continued data analysis and further potential collaborative investigations emerging from this project

Reportable Outcomes

- based on experience and training supported in this award, Dr Peaston's research assistant Tim Smith has applied for an Australian Postgraduate Award to support doctoral research.

Conclusion

In its second year, the project has again had setbacks delaying the planned molecular analyses. However, we can now move ahead methodically with molecular aspects of the project. We believe that the experimental replicates can be analysed as planned and substantially verified in the coming 12 months.